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Introduction

The E.Z.N.A.™ family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is Omega Bio-Tek's proprietary HiBind® matrix that avidly, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The E-Z 96®SE Plasmid Kit combines the power of HiBind® technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality plasmid DNA. The new E-Z 96® Lysate Clearance Plate obviates time-consuming centrifugation for clearing of the bacterial alkaline lysates. It also has an average DNA recovery rate 10 to 30% higher than the manual centrifuge method. Yields vary according to plasmid copy number, E.coli strain, and conditions of growth, but 1 mL of overnight culture in LB medium typically produces 10-15 µg high-copy plasmid DNA.

Storage and Stability

All E-Z 96® SE Plasmid Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: The mixture of Solution I/RNase A at 4°C; all other material at 22-25°C.

Kit Contents

Product Number	D1095-00	D1095-01	D1095-02
Purification times	1 x 96	4 x 96	20 x 96
96-Well 2ml Plate	1	4	20
E-Z 96® Lysate Clearance Plate	1	4	20
Solution I	40 mL	130 mL	3 x 200 mL
Solution II	40 mL	130 mL	3 x 200 mL
Neutralization Buffer	40 mL	130 mL	3 x 200 mL
RNase A, Concentrate	200 µL	600 µL	3 x 1 mL
SealPlate Sealing Film	6	24	2 x 60
Instruction Booklet	1	1	1

Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

Supplied By User:
Centrifuge capable of at least 3,000 x g.
Swinging-bucket rotor for 96-well plate
Sterile deionized water (or TE buffer)
96%-100% isopropanol
10-15 mL Culture tubes
70% ethanol

IMPORTANT	
	1. Add vial of RNase A to bottle of Solution I provided. Store at 4°C.
	2. Solution II should be kept at room temperature. Check before use for SDS precipitation, and if necessary redissolve SDS precipitate by warming. Close the Solution II bottle immediately after use to avoid the acidification of Solution II from CO_2 from air.

Note: All steps must be carried out at room temperature.

E-Z 96®SE Plasmid Isolation Vacuum Manifold Protocol

Supplied By User

- Centrifuge with swinging-bucket rotor at room temperature capable of 2000 x g
- Adapter for 96-well collection plate
- Vacuum pump or vacuum aspirator capable of achieving a vacuum of 20-24 inches Hg
- Standard vacuum manifold (i.e: Omega Product #VAC-03)
- Sterile deionized water
- Vacuum oven or incubator preset to 70°C

1. Culture Volume: **Innoculate 1.0-1.3 mL LB/antibiotic(s) medium placed in a 96-well 2mL culture block with *E.coli* carrying desired plasmid and grow at 37°C with agitation for 20-24 h.** It is strongly recommended that an endA negative strain of *E.coli* be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®.

2. Seal the plate with tape or film and pellet bacteria by **centrifugation at 1,500 x g for 5 minutes** in a swinging-bucket rotor at room temperature.

3. Remove the tape and discard supernatant into a waste container. Dry the plate by tapping the inverted block firmly a paper towel to remove excess media. **Add 300 µl Solution I/RNase A to the bacterial pellet in each well of the plate**. Resuspend cells completely by vortexing and/or pipetting.
   No cell clumps should be visible after resuspension of the pellet. Complete resuspension of cell pellet is vital for obtaining good plasmid yields.

4. **Add 300 µl Solution II into each well. Seal the 96-well Plate with a sheet of sealing film. Mix by gently shaking and rotating the plate for 1 minute to obtain a cleared lysate.** A 4 min incubation at room temperature may be necessary. The solution should become viscous and slightly clear. Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity. (Store Solution II tightly capped when not in use.)

5. **Remove the sealing film and add 300 µl of chilled (4°C) Neutralization Buffer into each well.** Dry the top of the plate with a paper tower. Seal the plate with sealing film and mix by gently inverting the plate for 10 times until a flocculent white precipitate forms.

6. **Optional:** Place the plate containing the cell lysate in a 92°C water bath for 8 minutes. This heating step denatures and precipitate the proteins and carbohydrates that are not removed by alkaline lysis. This heating step is essential for EndA$^+$ strains that normally have high level of endonuclease.

7. **Optional: Place the plate on ice and incubate for 10 minutes.**

8. Assemble the vacuum manifold: 1). Place E-Z 96® Lysate Clearance Plate in the top plate of manifold; 2). Place the 96-well 2 ml plate into the plate holder. 3). Place the top plate of manifold over the base, the 96-well 2 ml Plate now should be positioned under the E-Z 96® Lysate Clearance Plate. (Some manifolds might require internal height adjustment by using an extra small plate.) Seal the unused wells of E-Z 96® Lysate Clearance Plate with film tape.

9. Immediately transfer the lysate into the wells of E-Z 96® Lysate Clearance Plate. Allow the cell lysate to stand for 5 minutes. The white precipitate should float to the top.

10. Apply the vacuum until all the lysate passes through.

11. Turn off the vacuum and discard the E-Z 96® Lysate Clearance Plate. Add 650 ul of isopropanol to the cleared lysates in the 96-well plate, and seal well with sealing film. Immediately mix by inverting several times.

12. Centrifuge the 96-well 2 ml plate at 2,000 x g for 30 minutes at room temperature to precipitate the plasmid DNA. Discard the supernatant by inverting the block, and allow the 96-well 2 ml plate to remain inverted on a paper towel for 1-2 minutes.

13. Desalt the plasmid DNA by adding 600 ul of ice-cold 70% ethanol to each well, and seal each well with sealing film. Centrifuge at 2000 x g for 5 minutes. Discard the supernatant and invert the 96-well 2 ml plate on absorbent toweling to drain off residual solution. Dry the plasmid DNA for 15-20 minutes at room temperature or dry under vacuum for 10 minutes.

14. Add 40-80 ul of 1mM Tris-HCl, pH 8.5 buffer to each well, and seal each well with sealing film. Vortex to resuspend the plasmid DNA.

E-Z 96® SE Plasmid Isolation Spin Protocol

Supplied By User

- Centrifuge with swinging-bucket rotor at room temperature capable of 3,000 x g
- Adapter for 96-well collection plate
- Sterile deionized water
- Vacuum oven or incubator preset to 70°C

1. Culture Volume: **Innoculate 1.0-1.3 mL LB/antibiotic(s) medium placed in a 96-well 2mL culture block with *E.coli* carrying desired plasmid and grow at 37˚C with agitation for 20-24 h.** It is strongly recommended that an endA negative strain of *E.coli* be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®.

2. Seal the plate with tape or film and pellet bacteria by **centrifugation at 1,500 x g for 5 minutes** in a swinging-bucket rotor at room temperature.

3. Remove the tape and discard supernatant into a waste container. Dry the plate by tapping the inverted block firmly a paper towel to remove excess media. **Add 300 µl Solution I/RNase A to the bacterial pellet in each well of the plate**. Resuspend cells completely by vortexing and/or pipetting.

   No cell clumps should be visible after resuspension of the pellet. Complete resuspension of cell pellet is vital for obtaining good plasmid yields.

4. **Add 300 µl Solution II into each well. Seal the 96-well Plate with a sheet of sealing film. Mix by gently shaking and rotating the plate for 1 minute to obtain a cleared lysate.** A 4 min incubation at room temperature may be necessary. The solution should become viscous and slightly clear. Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity. (Store Solution II tightly capped when not in use.)

5. **Remove the sealing film and add 300 µl of chilled (4°C) Neutralization Buffer into each well.** Dry the top of the plate with a paper tower. Seal the plate with sealing film and mix by gently inverting the plate for 10 times until a flocculent white precipitate forms. Allow the 96-well 2 ml Plate to sit at room temperature for 5 minutes.

6. **Optional:** Place the plate containing the cell lysate in a 92°C water bath for 8

minutes. This heating step denatures and precipitate the proteins and carbohydrates that are not removed by alkaline lysis. This heating step is essential for EndA+ strains that normally have high level of endonuclease.

7. **Optional: Place the plate on ice and incubate for 10 minutes.**

8. Place the E-Z 96 Lysate Clearance Plate onto a clean 96-well 2ml plate. Using a pipetter, transfer the neutralized bacterial lysates from the 96-well 2 ml plate into the wells of the E-Z 96 Lysate Clearance Plate.

9. Place the E-Z 96 Lysate Clearance plate and the 96-well 2 ml plate together into the plate carrier. Spin at 2,000 x g for 5 minutes at room temperature.

10. Add 650 ul of isopropanol to the cleared lysates in the 96-well plate, and seal well with sealing film. Immediately mix by inverting several times.

11. Centrifuge the 96-well 2 ml plate at 2,500 x g for 30 minutes at room temperature to precipitate the plasmid DNA. Discard the supernatant by inverting the block, and allow the 96-well 2 ml plate to remain inverted on a paper towel for 1-2 minutes.

12. Desalt the plasmid DNA by adding 600 ul of ice-cold 70% ethanol to each well, and seal each well with sealing film. Centrifuge at 2,500 x g for 3 minutes. Discard the supernatant and invert the 96-well 2 ml plate on absorbent toweling to drain off residual solution. Dry the plasmid DNA for 15-20 minutes at room temperature or dry under vacuum for 10 minutes.

13. Add 30-80 ul of 1mM Tris-HCl, pH 8.5 buffer to each well, and seal each well with sealing film. Vortex to resuspend the plasmid DNA.

Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	Only use LB or YT medium containing Antibiotec(s). Do not use more than 2 mL with high copy plasmids. Cells may not be dispersed adequately prior to addition of Solution II. Vortex cell suspension to completely disperse. Increase incubation time with Solution II to obtain a clear lysate. Solution II if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS.
	Bacterial culture overgrown or not fresh.	Do not incubate cultures for more than 16 hr at 37°C. Storage of cultures for extended periods prior to plasmid isolation is detrimental.
	Low copy-number plasmid used	Such plasmids may yield as little as 0.1µg DNA from a 1 mL overnight culture. Increase culture volume to 3 mL.
High molecular weight DNA contamination of product.	Over mixing of cell lysate upon addition of Solution II.	Do not vortex or mix aggressively after adding Solution II. Adequate mixing is obtained by simply inverting and rotating tube to cover walls with viscous lysate.
Optical densities do not agree with DNA yield on agarose gel.	Trace contaminants eluted from column increase A_{260}.	Make sure to wash plate as instructed. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantization.
RNA visible on agarose gel.	RNase A not added to Solution I.	Add 1 vial of RNase to each bottle of Solution I.